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Deep Insight Section

Somatostatin (SS), SS receptors and SS analog treatment in tumorigenesis

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Abstract

Somatostatin (SS) is an inhibitory tetradecapeptide hormone with exocrine, endocrine, paracrine, and autocrine activities, which plays an important regulatory role in several cell functions, including inhibition of endocrine secretion and cell proliferation. Most of the effects of SS and of its currently available analogs are mediated via five different G protein-coupled receptor (GPCRs), codenamed sst_{1-5}. SS receptors (sst_s) are expressed in a tissue- and subtype-selective manner in both normal and neoplastic cells, and the majority of SS target tissues express multiple sst_s. Recent data suggest that when sst_s are coexpressed, they may interact forming homo- and hetero-dimers also with other GPCRs, thus altering their original pharmacological and functional profiles. The formation of dimers can be not only constitutive, but also ligand-promoted: hence, compounds with high affinity for the different receptor subtypes can be used to achieve effects elicited by specific dimers. A feature common to most GPCRs is the cyclic process of signaling, desensitization, internalization, resensitization, and recycling to the plasma membrane. These events prevent cells from undergoing excessive receptor stimulation or periods of prolonged inactivity. SS receptors differently internalize after agonist binding and, specifically, sst_2, sst_3 and sst_5 are internalized to a greater extent than sst_1 or sst_4. sst_s are linked to several second messenger systems which are involved in their downstream intracellular response (*i.e.*, adenylyl cyclase, calcium and potassium ion channels, Na^+/H^+ antiporter, phospholipase C, phospholipase A2, mitogen activated protein kinase, NO/cGMP, and serine-, threonine, and phosphotyrosyl- protein phosphatase).

Interestingly, SS and SS analogs can control tumor development and progression/metastatization by direct actions, mediated by the sst_s, and indirect actions, independent of receptor involvement. The direct antiproliferative effects include inhibition of autocrine/paracrine growth-promoting hormone/growth factor synthesis, arrest of cell division (by blockade of growth factor-mediated mitogenic signals), suppression of cell invasion and induction of apoptosis (programmed cell death). Indirect antitumor effects of SS include suppression of synthesis or/and release of growth factors and growth-promoting hormones, such as insulin, prolactin, insulin like-growth factor 1, epidermal growth factor, transforming growth factor-, gastrin, cholecystokinin and growth hormone. A specific pattern of sst_s activation thus seems to elicit relevant antitumoral actions and deserves further exploitation with the aim of validating novel therapeutic approaches to cancer.

1. Somatostatin

Somatostatin (SS) was first identified in the ovine hypothalamus as a tetradecapeptide that inhibited the release of growth hormone (Brazeau et al., 1973). SS-producing cells are present at high densities throughout the central and peripheral nervous systems. In the periphery, SS is also secreted by pancreas and gut and in a lesser extent by thyroid, adrenals and submandibular glands, kidneys, prostate, and placenta (Polak et al., 1975). SS mediates a variety of biological effects, the most

Signal peptide (24 aa)
met-leu-ser-cys-arg-leu-gln-cys-ala-leu-ala-ala-leu-ser-ile-val-leu-ala-leu-gly-cys-val-thr-gly

Propeptide (64 aa)
ala-pro-ser-asp-pro-arg-leu-arg-gln-phe-leu-gln-lys-ser-leu-ala-ala-ala-ala-gly-lys-gln-glu-leu-ala-lys-tyr-phe-leu-ala-glu-leu-leu-ser-glu-pro-asn-gln-thr-glu-asn-asp-ala-leu-glu-pro-glu-asp-leu-ser-gln-ala-ala-glu-gln-asp-glu-met-arg-leu-glu-leu-gln-arg

SS-28 (28 aa)
ser-ala-asn-ser-asn-pro-ala-met-ala-pro-arg-glu-arg-lys-ala-gly-cys-lys-asn-phe-phe-trp-lys-thr-phr-thr-ser-cys

SS-14 (14 aa)
ala-gly-cys-lys-asn-phe-phe-trp-lys-thr-phr-thr-ser-cys

Figure 1. Amino acid sequences of the human prosomatostatin.

important occurring at the pituitary (inhibition of growth hormone (GH) and tireotropic stimulating hormone (TSH) secretion) and gastroenteropancreatic (GEP) levels (inhibition of insulin, glucagon, and secretin secretion; inhibition of hydrochloric acid production and intestinal fluid absorption) (Konturek et al., 1976). In addition to inhibition of hormone secretion, SS also shows antiproliferative and anti-angiogenetic properties, that have been largely investigated both in cell lines (*i.e.*, human prostate cancer cells, human non small lung cell carcinomas and pituitary adenomas) and GH-secreting tumors. The SS form originally identified in the hypothalamus was SS-14, while SS-28, a congener of SS-14 extended at the N-terminus, was discovered subsequently (Shen et al., 1982). The single human SS gene is located on chromosome 3q28 and the correlate SS mRNA codes for a 116-amino acids (aa) prepro-SS protein (MW 12,727 Da). Prepro-SS has a sequence of hydrophobic aa at the N-terminus which is cleaved at the gly-ala junction at position -78 (from the N-terminus to the C-terminus). Pro-SS undergoes both monobasic (Arg^{-15}) and dibasic ($Arg^{-2}Lys^{-1}$) cleavages to release the two biofunctional hormones SS-28 and SS-14 (Funckes et al., 1983; Brakch et al., 2002) (Figure 1).

2. Somatostatin receptors

In mammals, the biological actions of SS are mediated by at least six G protein-coupled SS receptors (sst) encoded by five different genes, named sst_1-sst_5. Sst_2 exists in two splice variants, sst_{2A} (a long form) and sst_{2B} (a short form), which differ only in the length of the cytoplasmic tail. Sst_2 displays a cryptic intron at the 3' end of the coding region, which gives rise to the two spliced variants (Baumeister and Meyerhof, 2000; Olias et al., 2004). In the human gene, the spliced exon encodes for 25 aa residues compared to 38 residues in the unspliced form. The encoded receptor proteins range in size from 356 to 391 aa residues, showing the greatest sequence similarity in the putative transmembrane region, and diverge at their N- and C-terminal segments (Patel, 1999). Human sst_s genes are localized to chromosome 14q13 (sst_1), 17q24 (sst_2), 22q13.1 (sst_3), 20p11.2 (sst_4), and 16p13.3 (sst_5) (Yamada et al., 1993) encoding for proteins of 391 aa, 369 aa, 418 aa, 388 aa, and 363 aa, respectively (Yamada et al., 1992; Corness et al., 1993; Rohrer et al., 1993; Panetta et al., 1994). Structurally, those receptors belong to the so-called "superfamily" of G protein-coupled receptors (GPCRs). All sst isoforms possess a highly conserved sequence motif, *YANSCANPI/VLY*, in the seventh transmembrane region, which serves as a signature sequence for this receptor family (Kreienkamp et al., 2002). On the other hand, genes for $sst_{1, 3, 4}$, and sst_5 lack classical introns. Interestingly, the estimated sequence identity between sst_1 and sst_2 receptors is 46%. The deduced aa sequence of human sst_3 receptors displays the following degrees of similarity with other members of the sst family: 62% (sst_1), 64% (sst_2), and 58% (sst_4). Moreover, four sst_s have been identified in fish and variant forms of several sst_s also exist: sst_{3a}, sst_{3b}, sst_{5a}, sst_{5b}, and sst_{5c} in goldfish (Canosa et al., 2004), and sst_{1a} and sst_{1b} in trout (Slagter and Sheridan, 2004). As was the case with SS genes, phylogenetic analysis suggests that sst genes appear to have arisen from a series of gene duplication events.

2.1 Homo- and hetero-dimerization of somatostatin receptor subtypes

When sst_s in the cell membrane are coexpressed, they may interact forming homo- and hetero-dimers also with other GPCRs, thus altering their original pharmacological and functional profiles. A series of studies, carried out on transfected cell lines, have shown that dimers can consist of two identical sst subtypes (homodimers) or two different subtypes (heterodimers), with a range of possible combinations depending on the specific subtype and, probably, on the specific sst-expressing population (Baragli et al., 2007). The five SS receptor isoforms can be involved in the formation of different dimers, namely, sst_1 and sst_5 bind efficiently together, while stable sst_4-sst_5 dimers have not been observed. These interactions are capable to provide greater signalling diversity, affecting the downstream intracellular effects mediated by receptor activation, such as ligand binding affinity, agonist-induced regulation and trafficking. In fact, sst_1

endocytosis is enhanced when sst_1 and sst_5 are co-expressed in the same cell and sst_5 is activated; conversely, the internalization of sst_2 is delayed by sst_5 and sst_2 co-expression. Moreover, sst_s can form also heterodimers with other GPCRs: sst_2 interacts with the μ-opioid receptor, and sst_5 binds to the D_2 dopamine receptor (D_2R). Interestingly, the sst_5-D_2R dimer enhances the effects of both receptors, leading to a more potent inhibition of adenylyl cyclase (AC) (Møller et al., 2003).

The dimer formation can be not only constitutive, but also ligand-promoted: hence, compounds with high affinity for the different receptor subtypes can be used to achieve effects elicited by specific dimers. In the last years, a variety of mono-, bi- and pan-specific SS analogs has been synthesized, allowing the characterization of the intracellular effectors involved in the downstream signalling of the different sst_s (Saveanu et al., 2001). The new receptor specific compounds showed to be useful under many aspects; among them, the understanding of the synergistic effect caused by the simultaneous activation of different receptors. In cultured pituitary cells, a sst_2-D_2R chimeric compound (BIM-23A387) showed a more potent action in inhibiting prolactin (PRL) and GH secretion compared to the related mono-specific analogs, either alone or in combination (Ferone et al., 2007). A similar pattern of action has been observed for the anti-secretory and anti-proliferative activity in prostate and lung *in vitro* models, where the treatment with the chimeric sst_2-sst_5 and sst_2-sst_5-D_2R compounds were more effective than the respective mono-specific SS and D_2R analogs (Arvigo et al., 2010). This evidence suggests that the concurrent activation of different GPCRs triggers their dimerization, leading to an enhanced effect.

2.2 Trafficking of somatostatin receptor subtypes

A feature common to most GPCRs is the cyclic process of signaling, desensitization, internalization, resensitization, and recycling to the plasma membrane. These events prevent cells from undergoing excessive receptor stimulation or periods of prolonged inactivity (van Koppen et al., 2004).

SS receptors differently internalize after agonist binding and, specifically, sst_2, sst_3 and sst_5 are internalized to a higher extent than sst_1 or sst_4. Among all subtypes, the agonist-mediated trafficking of both sst_2 splicing isoforms are the mostly described (Jacobs and Schulz, 2008). Investigations in neuroendocrine tumors showed that both sst_{2A} and sst_{2B} isoforms are rapidly desensitized and internalized after agonist-mediated phosphorylation. Receptor phosphorylation, which involves sites located in the third intracellular loop and in the C-terminal tail, is followed by recruitment of β-arrestin to the receptor forming a stable complex, which is internalized into the same endocytotic vesicles. Interestingly, the binding affinity of the agonist plays an important role in the degree of receptor internalization. A high binding affinity of the agonist is a prerequisite for triggering sst_2 internalization. In fact, the bi-specific sst_2/sst_5 analog BIM23244, which has a greater sst_2 affinity compared to L-817/818 analog is able to induce a greater internalization (Jacobs and Schulz, 2008).

Sst_5 differs from sst_{2A} in its cellular localization and appears to be predominantly located in intracellular components even without agonist treatment, whereas after stimulation, a large amount of intracellular receptors is recruited to the cell surface. The sst_5 third intracellular (i3) loop and the C-terminal tail have been found to regulate receptor internalization, which occurs via clathrin-dependent mechanisms. In cultured pituitary cell lines, where sst_5 underwent different kinds of point mutations within the i3 loop, there is a reduction of receptor internalization upon SS-28 treatment. Moreover, by using different C-terminus truncated forms of the receptor, an enhanced sst_5 internalization has been observed, thus showing that the sst_5 C-terminal tail, or at least a part of it, has an inhibitory role in receptor internalization (Peverelli et al., 2008).

Sst_3, which shows high affinity for SS-14, internalizes efficiently after agonist stimulation through a clathrin-dependent mediated pathway. Without stimulation, sst_3 is almost exclusively located at the plasma membrane, whereas after agonist withdrawal only a small amount of sst_3 is recycled to the cell surface (Peverelli et al., 2008).

Hence, due to the differential expression of SS receptors in tumors, the comparison of their ability to undergo agonist-induced desensitization and internalization may provide important clues for the clinical use of SS analogs. In this context, an *in vitro* study demonstrated that short-term administration of the multiligand (sst_1/sst_2/sst_3/sst_5) pasireotide (SOM230) modulates SS receptor trafficking in a manner clearly distinct from octreotide (sst_2/sst_5) (Tulipano and Schulz, 2007). SOM230 was less potent than octreotide in inducing signaling and internalization of the sst_2 receptor. Whereas octreotide-activated sst_2 receptors cointernalized with β-arrestin-2 into the same endocytic vesicles, SOM230-mediated sst_2 activation led to the formation of unstable complexes that dissociated at or near the plasma membrane. Sst_2 receptors recycled faster to the plasma membrane in SOM230- than in octreotide-treated cells. The accelerated recycling of SOM230-activated receptors may counteract homologous desensitization in sst_2-expressing cells and, hence, result in longer lasting functional responses of SOM230 (Lesche et al., 2009).

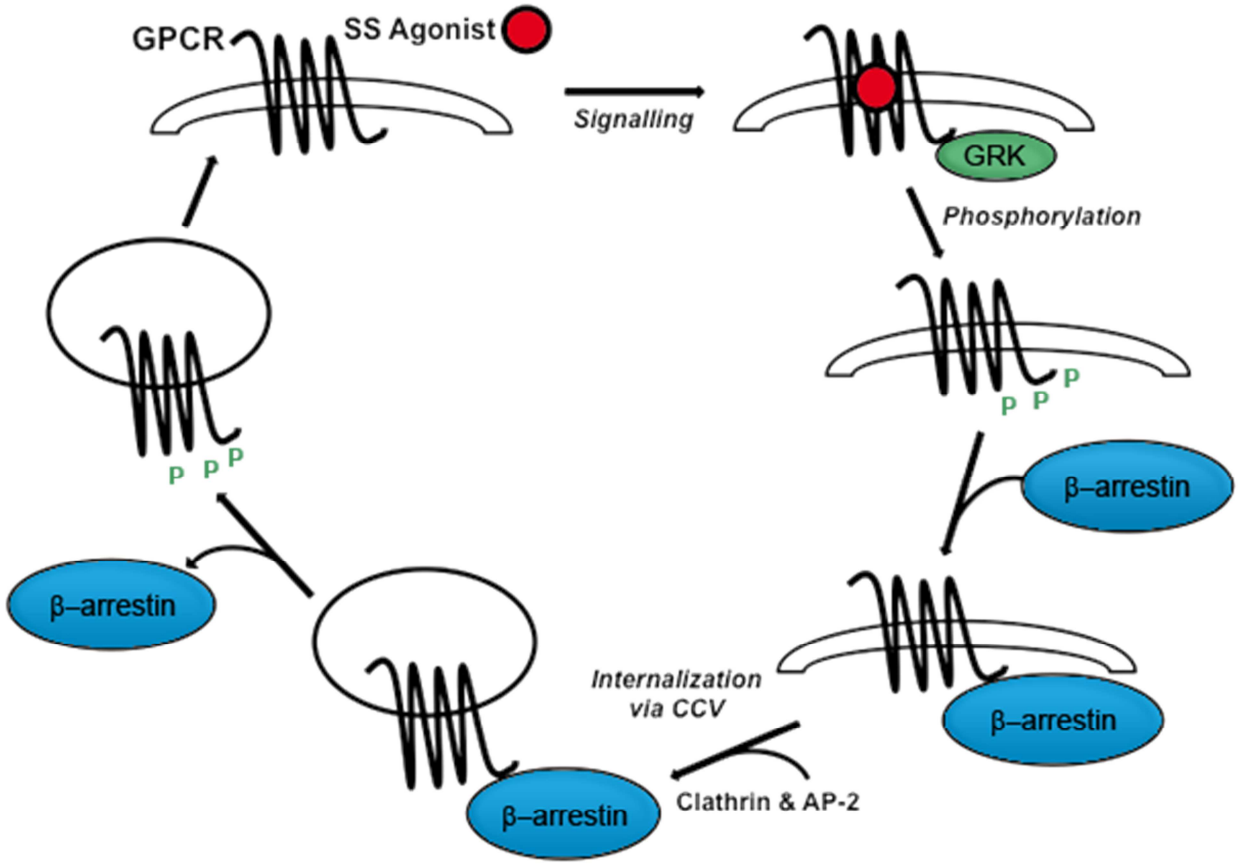

Figure 2. Schematic representation of a ligand-driven somatostatin receptor internalization. GRK: GPCR kinase; CCV: clathrin-coated vesicle (modified from van Koppen et al., 2004).

2.3 Somatostatin receptor signalling pathways

All five SS isoforms (sst_s) bind/interact to G proteins to activate their signalling pathways. They couple to all three G_i subunits (G_{i1}, G_{i2}, and G_{i3}) leading to a potent inhibition of AC activation, and then of cyclic AMP (cAMP) synthesis. Specifically, sst_1 is coupled to AC via G_{i3}; sst_{2A} is able to associate with G_{i1}, G_{i2}, G_{i3}, and Ga_{o2}; sst_3 interacts with G_{i1}, G_{i2}, G_{i4}, and G_{i6} (Reisine and Bell, 1995).

Several second messenger systems are involved in their downstream intracellular response: AC, calcium (Ca^{2+}) and potassium (K^+) ion channels, sodium (Na^+)/H^+ antiporter, phospholipase C (PLC), phospholipase A2 (PLA2), mitogen activated protein kinase (MAPK), NO/cGMP, and serine-, threonine-, and phosphotyrosyl-protein phosphatase (PTP) (Patel, 1999).

Sst_2 and sst_4 are the main receptors that activate voltage-gated K^+ current (Yang and Chen, 2007). As a result of their activation, membrane hyperpolarization occurs, hindering any subsequent spontaneous membrane potential and leading to a reduction in intracellular Ca^{2+}. Sst_s can differently modify Ca^{2+} currents; in AtT-20 murine cell line, both sst_2 and sst_5 can couple negatively to an L-type Ca^{2+} channel reducing Ca^{2+} influx (Tallent et al., 1996), whereas, conversely, in the GH3 rat pituitary tumor cell line, only sst_2 blocks voltage-gated Ca^{2+} current (Yang and Chen, 2007).

The human sst_s also stimulate PTP through a pertussis toxin-sensitive pathway involving G_{i2}, but differencies among the various species have been found, since sst_5 in rat does not regulate PTP. The first evidence of sst_s-mediated activation of PTP was given by the counteraction driven by sst_s on tyrosine kinase receptors-mediated proliferative effect (Florio, 2008a). One of the main downstream effects of sst_s-mediated PTP activation is the inhibition of MAPK ERK1/2 activity. Several data exist about the inhibition of the MAPK signalling cascade by three of the five sst subtypes: sst_2, sst_3 and sst_5. In AtT-20 and in transfected CHO-K1 cells, sst_5 constitutively restrains ERK1/2 phosphorylation (Ben-Shlomo et al., 2007), and sst_2 and sst_3 mediated the same inhibitory signal in SHSY-5Y neuroblastoma cells and in NIH3T3 cells, respectively. Conversely, sst_1 and sst_4 stimulate the MAPK pathway (Patel, 1999).

Glutamate receptor ion channels are also involved in sst_s signalling: sst_2 inhibits AMPA/kainate receptor-mediated glutamate currents, while sst_1 stimulates AMPA/kainate receptor activity in cultured mouse hypothalamic neurons.

Inositol 1,4,5-trisphosphate (IP3) represents another intracellular signalling pathway linked to sst_2. In CHO-DG44 cells, it takes place via sst_2-mediated activation of phosphatidylinositol 3-kinases (PI3K), whereas in astrocytes and in intestinal smooth muscle cells it is driven by PLC (Florio, 2008a). Experimental data in rat pituitary F4C1 cells indicate that the activation of sst_2, but not sst_1, stimulates PLC activity and increases cytosolic Ca^{2+}level, due to Ca^{2+} release from intracellular stores (Rosskopf et al., 2003).

In hippocampal neurons, SS effect on PLA-2-dependent stimulation of arachidonate production has been associated with sst_4, which is able to elicit arachidonate synthesis through phospholipase A2 (PLA-2) activation (Patel, 1999).

In colon carcinoma, enteric endocrine and hepatic cells, the Na^+/H^+ exchangers can be also activated by sst_1, sst_3 and sst_4, but not by sst_2 and sst_5 (Florio, 2008a).

Interestingly, in human sst_5 there are two regions, the BBXXB domain and the DRY motif, located in the third intracellular (i3) and second intracellular (i2) loops, respectively, which are needed to activate the signalling pathways mediated by this receptor subtype. Namely, the BBXXB domain, although being required in the subtype 5 downstream effectors generation, is not directly involved in interactions with G_i protein, since a mutation in the first BBXXB residue does not affect the receptor ability of inhibiting cAMP accumulation. Conversely, the DRY motif was found to be crucial in coupling with G_i protein, since mutations in the DRY sequence do not impair sst_5-driven inhibition of cAMP production. However, both regions are necessary to mediate the other sst_5 intracellular responses, such as cytoplasmic Ca^{2+} reduction and inhibition of ERK1/2 phosphorylation (Peverelli et al., 2009).

3. Tumorigenesis

Tumorigenesis is a collection of complex genetic diseases characterized by multiple defects in the homeostatic mechanisms that regulate cell growth, proliferation and differentiation. In humans, several lines of evidence indicate that tumorigenesis is a multistep process which reflects genetic alterations that drive the progressive transformation of normal cells into highly malignant derivatives. Tumorigenesis is thought to require four to six stochastic rate-limiting mutation events to occur in the lineage of one cell. Hanahan and Weinberg (Hanahan and Weinberg, 2000) suggest that six cellular alterations, or hallmarks, collectively drive a population of normal cells to become a cancer. The six hallmarks are (i) self-sufficiency in growth signals (SG), (ii) insensitivity to antigrowth signals (IA), (iii) evasion of apoptosis (EA), (iv) limitless replicative potential (LR), (v) sustained angiogenesis (SA), and (vi) tissue invasion and metastasis. Genetic instability (GI) is defined as an "enabling characteristic" that facilitates the acquisition of other mutations due to defects in DNA repair. These hallmarks form a candidate set of rules that underlie the transformation of a normal tissue to a cancerous one and are shared in common by most and perhaps all types of human tumors (Spencer et al., 2006).

3.1 Antitumor actions of somatostatin and somatostatin analogs

SS has been shown to display several biological actions which include inhibition of exocrine and endocrine secretions, gut motility, cell proliferation, cell survival and angiogenesis. SS analogs show antineoplastic and antiproliferative activity in many experimental *in vivo* and *in vitro* models and this activity is principally attributed to activation of sst_2 and sst_5. SS analogs treatment can be effective in the control of tumor growth in humans and in 37-82% of patients receiving SS analogs, as primary medical therapy, tumor shrinkage has been observed. The antiproliferative and antitumoral effects of SS analogs occur independently of their antisecretory and antihormonal effects. From these results we can infer that antisecretory and antitumor effects of SS and SS analogs are mediated by different receptors/signalling pathways and that the antiproliferative effect of these synthetic compounds may depend on tumor sst_s profile, but also on the specific target cell intracellular signalling (Pyronnet et al., 2008).

SS and SS analogs can control tumor development and progression/metastatization by two separate mechanisms: direct actions, mediated by the sst_s, and indirect actions, independent of the receptors.

3.1.1 Direct somatostatin antitumor actions

Direct effects of SS and its analogs on tumor cell growth and spread derive from interaction with specific tumor cell membrane receptors. The direct antiproliferative actions include inhibition of autocrine/paracrine growth-promoting hormone/growth factor synthesis, arrest of cell division (by blockade of growth factor-mediated mitogenic signals), suppression of cell invasion and induction of apoptosis (programmed cell death) (Pyronnet et al., 2008). The exact antitumoral mechanism initiated by SS analogs depends on the tumor cell type and the sst_s to which it binds. In this way, each receptor subtype is able to mediate different biological actions (Susini and Buscail, 2006).

Cell cycle arrest is mediated by interaction of SS with its five receptors and the consequent initiation of several intracellular signalling pathways, which are either activated or inhibited according to the sst subtype, the downstream recruited enzyme and cell environment. These pathways include activation of tyrosine kinases (JAK, c-src) and tyrosine phosphatases (SHP1, SHP2, PTP), activation/inhibition of nitric oxide synthase/cGMP-dependent protein kinase, Ras/ERK pathway and inhibition of PI3 kinase/Akt pathway, which in turn lead to induction of the cyclin-dependent kinase inhibitor $p27^{kip1}$ or $p21^{Cip1}$ and cell cycle arrest (Pyronnet et al., 2008). SS also induces cell growth inhibition through restoration of functional gap

junctions. These structures are composed of connexins and play a pivotal role in maintaining the differentiated state and cell-contact inhibition. Actually, in most cancer cells, it has been observed an impaired expression of connexins (Lahlou et al., 2005). It has been demonstrated that SS is also a potent anti-migratory and anti-invasive agent for various tumor cells. Inhibition of cell invasion occurs through molecular mechanisms which are cell type specific and depend on sst expression pattern, on sst effector coupling as well as on the signalling cascade involved in target cells (Pola et al., 2003).

SS analogs are also thought to inhibit cell proliferation by inducing apoptosis. The receptor subtypes primarily involved in SS-induced apoptosis are sst_3 and sst_2. Apoptotic effect is achieved by regulation of the two main signalling pathways, the cell-extrinsic pathway (triggered by death receptors) and the cell-intrinsic pathway (also called the mitochondrial pathway) (Pyronnet et al., 2008; Florio, 2008b).

SS and its chemically designed analogs are potential therapeutic agents, in particular for the treatment of endocrine diseases that cause hormone hypersecretory syndromes. SS and its commercially available analogs exert antisecretory and antiproliferative effects by interacting with one or more of the five sst_s, which then trigger various intracellular signalling pathways according to the tissue, thus possibly leading to different actions. The tissue expression patterns of sst_s, the binding profile of agonists and sst_s effector coupling confer functional and therapeutic specificity to ligand activity (Zatelli and degli Uberti, 2009).

The two SS analogs currently used in the clinics are Octreotide and Lanreotide. They have demonstrated efficacy in reducing GH and IGF-1 levels in up to 60% of patients with acromegaly and therefore have been widely used in the treatment of GH hypersecretion (Shimon et al., 1997). The main pharmacological target of these compounds is sst_2, the receptor subtype which is the most frequently expressed in human GH-secreting pituitary adenomas, but they also bind, with a lesser affinity, to sst_5. However, a significant proportion of patients with acromegaly is resistant to the treatment with ocreotide.

Pasireotide (SOM-230), a compound that interacts with multiple sst_s ($sst_{1\text{-}2\text{-}3\text{-}5}$) is able to inhibit GH secretion in octreotide-resistant pituitary adenomas, representing a potential therapy for octreotide-resistant acromegaly patients (Petersenn et al., 2010). The efficacy of pasireotide in overcoming octreotide resistance has been attributed to its ability of binding to all sst_s and, in particular, to its greater affinity for sst_5, which is up-regulated in such tumors. This sst_s multiligand compound showed *in vitro* a significant reduction of cell viability in many non-functioning pituitary adenomas (NFPAs) probably through the inhibition of VEGF secretion. Several results suggested that pasireotide could be a potential therapeutic agent for conditions characterized by an excess of ACTH. In patients with Cushing's disease, the administration of pasireotide decreased urinary free cortisol levels and significantly improved symptoms associated with the disease. Moreover, in ACTH-secreting pituitary tumor cells, pasireotide reduced ACTH secretion and cell proliferation (Bode et al., 2010). NFPAs represent a possible therapeutic target also for selective sst_1 agonists as these tumors have been demonstrated to express sst_1. The sst_1 agonist BIM-23926 has exhibited antisecretory and antiproliferative effects in a group of NFPAs *in vitro*. Moreover, several findings support the hypothesis that chimeric sst_s/DR agonists can be effective in suppressing in vitro cell proliferation in the majority of NFPAs. Indeed, BIM-23A387, a chimeric sst_2/DR_2 selective agonist inhibits cell viability in most NFPA primary cultures, as well as BIM-23A760, a compound with high affinity for DR_2, sst_2 and sst_5 significantly suppresses DNA synthesis in the 60% of the NFPA cultures tested (Florio et al., 2008).

It has been observed that SS and its analogs can decrease plasma calcitonin levels and improve symptoms in patients with medullary thyroid carcinoma (MTC), but their antiproliferative effects remain controversial. As a matter of fact, in TT cells, a human MTC cell line expressing all sst_s, sst_2 activation leads to inhibition of DNA synthesis and cell proliferation, whereas sst_5 activation has an opposite effect. Thus, we can infer that sst_2 and sst_5 agonists can antagonize the activity of one another in contrast to what happens in pituitary adenomas. Potent sst_1-selective ligands (BIM-23296 and BIM-23745) could have a therapeutic role in MTC because they are effective in reducing DNA synthesis, the viability of TT cells, calcitonin secretion and gene expression (Zatelli et al., 2006).

Sst_s are also highly expressed in most neuroendocrine tumors with a variable expression patterns. Treatment with Octreotide and Lanreotide is ineffective in inhibiting hormone secretion in some patients with neuroendocrine tumors because they develop tachyphylaxis. Conversely, pasireotide has shown a considerable reduction of symptoms in the majority of patients with metastatic gastroenteropancreatic endocrine tumors (Desai et al., 2009).

Experimental data on prostate cancer showed that four ($sst_{1\text{-}2\text{-}3\text{-}5}$) out of five sst_s receptors were found to be expressed in the LNCaP cell line, an *in vitro* model of human androgen-dependent PCa. Their activation by selective SS agonists resulted in a significant anti-proliferative effect with a peculiar pattern according to receptor subtype, ligand affinity and, possibly, receptor dimerization. Moreover, such treatments were also able to modulate the profile of the IGF system, known to be involved in PCa progression. Interestingly, these data provide strong evidence for an inhibitory role of sst_1 activation on PCa cell proliferation, suggesting that SS agonists with enhanced sst_1 affinity and selectivity may have great potentiality as pharmacological tools for at least androgen-dependent PCa treatment. In addition, the antiproliferative effect of sst_1 and sst_5 mono-

specific agonists may be due, at least in part, to the inhibition of IGF-I secretion (Ruscica et al., 2010).

3.1.2 Indirect somatostatin antitumor mechanisms

Indirect antitumor effects of SS include suppression of synthesis or/and release of growth factors and growth-promoting hormones such as insulin, prolactin, IGF-1, epidermal growth factor (EGF), transforming growth factor- (TGF-), gastrin, cholecystokinin (CCK) and GH.

Several experimental *in vitro* and *in vivo* results indicate that another indirect action of SS and SS analogs on tumor growth may be the inhibition of angiogenesis. Angiogenesis, the formation of new blood vessels from an existing capillary network, is necessary for tumor neovascularization, which is essential for tumor growth, invasion and for dissemination of metastasis. By limiting the blood supply, tumor growth can be effectively controlled (Kvols and Woltering, 2006). SS and SS analogs exert antiangiogenic actions through different mechanisms like suppression of endothelial cell proliferation and arrest of monocyte and endothelial cell migration. Normal endothelial cells lack sst_2 receptors and the expression of this receptor subtype on endothelial cells uniquely appears as they proliferate to form new blood vessels (Kvols and Woltering, 2006). So, the inhibition of angiogenesis may result from the up-regulation of sst_2 during the angiogenic switch from resting to proliferating endothelium. However, other sst_s such as sst_3 and sst_5 may also play a role. At the molecular level, this effect results from SS-mediated inhibition of MAP kinase activity and endothelial NO synthase (eNOS) activity. Another mechanism by which SS analogs suppress angiogenesis is through a broad inhibition of both the release and the effect of growth factors, some of which are angiogenic, including vascular endothelial growth factor (VEGF), platelet-derived growth factor, IGF-1 and basic fibroblast growth factor. These growth factors, secreted by tumor cells and infiltrating inflammatory cells, stimulate endothelial and smooth muscle cell proliferation and migration, important processes in angiogenesis (Zatelli et al., 2007).

4. Somatostatin receptor activation and tumorigenesis: future directions

According to the evidence reported in the present paper, a specific pattern of sst_s activation seems to elicit important antitumoral actions with potential relevance to some solid tumors expressing these receptor isoforms. In addition to the well-established anti-secretory effects, which may affect the cancer-associated paraneoplastic syndrome as well as the possible autotrophic actions of tumor-produced secretory proteins, a consistent body of data indicates that stimulation of tumor-expressed sst_s results in a multi-step restrain of tumorigenesis. These mechanisms thus deserve further exploitation with the aim of validating novel therapeutic approaches to cancer.

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